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(54) Title: CYTOKINE RECEPTOR CHAIN

(57) Abstract: Polynucleotides encoding the IL-13 receptor and fragments thereof are disclosed. IL-13 receptor proteins, methods for their production, inhibitors of binding of IL-13 and its receptor and methods for their identification are also disclosed. Methods of medical treatment using such molecules and antagonists of the IL-13/IL-13R interaction are also provided.

# CYTOKINE RECEPTOR CHAIN

This application is a continuation-in-part of application Ser. No. 08/841,751, filed April 30, 1997, which was a divisional application of application Ser. No. 08/609,572, filed March 1, 1996.

## Field of the Invention

The present invention relates to mammalian cytokine receptor proteins with affinity for IL-13 (including without limitation human and murine receptor proteins), fragments thereof and recombinant polynucleotides and cells useful for expressing such proteins.

## Background of the Invention

A variety of regulatory molecules, known as cytokines, have been identified including interleukin-13 (IL-13). Various protein forms of IL-13 and DNA encoding various forms of IL-13 activity are described in McKenzie et al., Proc. Natl. Acad. Sci. USA 90:3735 (1993); Minty et al., Nature 362:248 (1993); and Aversa et al., WO94/04680. Thus, the term "IL-13" includes proteins having the sequence and/or biological activity described in these documents, whether produced by recombinant genetic engineering techniques; purified from cell sources producing the factor naturally or upon induction with other factors; or synthesized by chemical techniques; or a combination of the foregoing.

IL-13 is a cytokine that has been implicated in production of several biological activities including: induction of IgG4 and IgE switching, including in human immature B cells (Punnonen et al., J. Immunol. 152:1094 (1994)); induction of germ line IgE heavy chain ($\epsilon$) transcription and CD23 expression in normal human B cells (Punnonen et al., Proc. Natl. Acad. Sci. USA 90:3730 (1993)); and induction of B cell proliferation in the presence of CD40L or anti-CD40 mAb (Cocks et al., Int. Immunol. 5:657 (1993)). Although many activities of IL-13 are similar to those of IL-4, in contrast to IL-4, IL-13 does not have growth promoting

effects on activated T cells or T cell clones (Zurawski et al., EMBO J. 12:2663 (1993)).

Like most cytokines, IL-13 exhibits certain biological activities by interacting with an IL-13 receptor ("IL-13R") on the surface of target cells. IL-13R and the IL-4 receptor ("IL-4R") sharing a common component, which is required for receptor activation; however, IL-13 does not bind to cells transfected with the 130 kD IL-4R (Zurawski et al., *supra*). Thus, the IL-13R must contain at least one other ligand binding chain. Cytokine receptors are commonly composed or two or three chains. The cloning of one ligand binding chain for IL-13 has been recently reported (Hilton et al., Proc. Natl. Acad. Sci. 93:497-501).

It would be desirable to identify and clone the sequence for any other IL-13 binding chain of IL-13R so that IL-13R proteins can be produced for various reasons, including production of therapeutics and screening for inhibitors of IL-13 binding to the receptor and receptor signaling.

Summary of the Invention

In accordance with the present invention, polynucleotides encoding the IL-13 binding chains of the interleukin-13 receptor are disclosed, including without limitation those from the murine and human receptors. In certain embodiments, the invention provides an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 256 to nucleotide 1404;

(b) the nucleotide sequence of SEQ ID NO:3 from nucleotide 103 to nucleotide 1242;

(c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) or (b) as a result of degeneracy of the genetic code;

(d) a nucleotide sequence capable of hybridizing under stringent conditions to the nucleotide specified in (a) or (b);

(e) a nucleotide sequence encoding a species homologue of the sequence specified in (a) or (b); and

(f) an allelic variant of the nucleotide sequence specified in (a) or (b). Preferably, the nucleotide sequence encodes a protein having a biological activity of the human IL-13 receptor. The nucleotide sequence may be operably linked to an expression control sequence. In preferred embodiments, the polynucleotide comprises the nucleotide sequence of SEQ ID NO:1 from nucleotide 256 to nucleotide 1404; the nucleotide sequence of SEQ ID NO:1 from nucleotide 319 to nucleotide 1257; the nucleotide sequence of SEQ ID NO:1 from nucleotide 1324 to nucleotide 1404; the nucleotide sequence of SEQ ID NO:3 from nucleotide 103 to nucleotide 1242; the nucleotide sequence of SEQ ID NO:3 from nucleotide 178 to nucleotide 1125; or the nucleotide sequence of SEQ ID NO:3 from nucleotide 1189 to nucleotide 1242.

The invention also provides isolated polynucleotides comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 334;

(c) the amino acid sequence of SEQ ID NO:2 from amino acids 357 to 383;

(d) the amino acid sequence of SEQ ID NO:4;

(e) the amino acid sequence of SEQ ID NO:4 from amino acids 26 to 341;

(f) the amino acid sequence of SEQ ID NO:4 from amino acids 363 to 380; and

(g) fragments of (a)-(f) having a biological activity of the IL-13 receptor binding chain. Other preferred embodiments encode the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 331 and the amino acid sequence of SEQ ID NO:2 from amino acids 26 to 331.

Host cells, preferably mammalian cells, transformed with the polynucleotides are also provided.

In other embodiments, the invention provides a process for producing a IL-13bc protein. The process comprises:

(a) growing a culture of the host cell of the present invention in a suitable culture medium; and

(b) purifying the human IL-13bc protein from the culture.

Proteins produced according to these methods are also provided.

The present invention also provides for an isolated IL-13bc protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 334;

(c) the amino acid sequence of SEQ ID NO:2 from amino acids 357 to 383;

(d) the amino acid sequence of SEQ ID NO:4;

(e) the amino acid sequence of SEQ ID NO:4 from amino acids 26 to 341;

(f) the amino acid sequence of SEQ ID NO:4 from amino acids 363 to 380; and

(g) fragments of (a)-(f) having a biological activity of the IL-13 receptor binding chain

Preferably the protein comprises the amino acid sequence of SEQ ID NO:2; the sequence from amino acid 22 to 334 of SEQ ID NO:2; the sequence of SEQ ID NO:4; or the sequence from amino acid 26 to 341 of SEQ ID NO:4. In other preferred embodiments, the specified amino acid sequence is part of a fusion protein (with an additional amino acid sequence not derived from IL-13bc). Preferred fusion proteins comprise an antibody fragment, such as an Fc fragment. Particularly preferred embodiments comprise the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 331 and the amino acid sequence of SEQ ID NO:2 from amino acids 26 to 331.

Pharmaceutical compositions comprising a protein of the present invention and a pharmaceutically acceptable carrier are also provided.

The present invention further provides for compositions comprising an antibody which specifically reacts with a protein of the present invention.

Methods of identifying an inhibitor of IL-13 binding to the IL-13bc or IL-13 receptor are also provided. These methods comprise:

(a) combining an IL-13bc protein or a fragment thereof with IL-13 or a fragment thereof, said combination forming a first binding mixture;

(b) measuring the amount of binding between the protein and the IL-13 or fragment in the first binding mixture;

(c) combining a compound with the protein and the IL-13 or fragment to form a second binding mixture;

(d) measuring the amount of binding in the second binding mixture; and

(e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture;

wherein the compound is capable of inhibiting IL-13 binding to the IL-13bc protein or IL-13 receptor when a decrease in the amount of binding of the second binding mixture occurs. Inhibitors of IL-13R identified by these methods and pharmaceutical compositions containing them are also provided.

Methods of inhibiting binding of IL-13 to the IL-13bc proteins or IL-13 receptor in a mammalian subject are also disclosed which comprise administering a therapeutically effective amount of a composition containing an IL-13bc protein, an IL-13bc or IL-13R inhibitor or an antibody to an IL-13bc protein.

Methods are also provided for potentiating IL-13 activity, which comprise combining a protein having IL-13 activity with a protein of claim 11 and contacting such combination with a cell expressing at least one chain of IL-13R other than IL-13bc. Preferably, the contacting step is performed by administering a therapeutically effective amount of such combination to a mammalian subject.

Further methods are provided for treating an IL-13-related condition in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition comprising an IL-13 antagonist and a pharmaceutically acceptable carrier. Other methods provide for a method of inhibiting the interaction of IL-13 with an IL-13bc protein in a mammalian subject

comprising administering a therapeutically effective amount of a composition comprising an IL-13 antagonist and a pharmaceutically acceptable carrier. Preferably, the antagonist is selected from the group consisting of an IL-13bc protein, a soluble form of IL-13Rα1, an antibody to IL-13 or an IL-13-binding fragment thereof, an antibody to IL-13bc or an IL-13bc-binding fragment thereof, an antibody to IL-13Rα1 or an IL-13Rα1-binding fragment thereof, IL-13R-binding mutants of IL-4, a small molecule capable of inhibiting the interaction of IL-13 with IL-13bc and a small molecule capable of inhibiting the interaction of IL-13 with IL-13Rα1.

Brief Description of the Figure

Fig. 1: The figure presents photographs of IL-13, IL-4, IL-11 and mock transfected COS cells after exposure to IL-13bc-Fc as described in Example 4 below.

Fig. 2: Reversal of allergen-induced airway hyper responsiveness by in vivo blockade of interleukin-13. 10 days after initial intratracheal challenge, OVA- and PBS-immunized mice were again challenged intratracheally with either OVA or PBS. Mice were given sIL-13bc-Fc (400ug) or an equivalent amount of control hu-IgG by intraperitoneal injection on Day -1, O, +1 and +3 of the secondary antigen challenge. The allergic phenotype was assessed 4 days after the PBS or OVA challenge. (A) Airway hyper responsiveness (AHR) to acetylcholine challenge, defined by the time-integrated rise in peak airway pressure (airway-pressure-time index [APTI] in $cmH_2O$ x sec). (B) Inflammatory cell composition of bronchoalveolar lavage fluids. Cell differential percentages were determined by light microscopic evaluation of cytospin preparations. Data are expressed as absolute numbers of cells. (C) OVA-specific serum IgE concentrations. Results are means +/- SEM of 8-10 animals per group. $*P < 0.05$ compared with respective PBS control groups; $**P < 0.05$ compared to OVA/Hu-Ig group (one-way ANOVA followed by Fisher's least significant difference test for multiple comparisons).

Fig. 3: Effects of IL-13 blockade on allergen-driven increases in mucus-containing cells in the airway epithelium. Lung sections (N = 4 per experimental

group, four sections per animal) were fixed in formalin, cut into 10um sections and stained with hematoxylin and eosin, and periodic acid Schiff. Representative sections are shown. Bars =100 um. PBS/Hu-Ig: PBS-immunized and challenged controls, demonstrating few mucus-containing cells. OVA/Hu-Ig: allergen-induced increases in interstitial inflammatory cells and increases in the number of goblet cells containing mucus. OVA/sIL-13bc-Fc: dramatic inhibitory effect of IL-13 blockade on allergen-induced goblet cell mucus production.

Fig. 4: IL-13 induction of airway hyperreactivity. Naive mice were given recombinant IL-13 (5 ug/mouse, 50 ul volume) or PBS daily by intratracheal instillation. 24 hrs after the last treatment, (A) Airway hyper responsiveness, (B) BAL eosinophil levels, (C) Serum total IgE levels, and (D) Mucus score were determined. Results are means +/- SEM (vertical bars) of 7-10 animals per group. $*P < 0.05$ compared to PBS group (Student's t test).

Detailed Description of Preferred Embodiments

The inventors of the present application have for the first time identified and provided polynucleotides encoding the IL-13 binding chain of IL-13R (hereinafter "IL-13bc"), including without limitation polynucleotides encoding murine and human IL-13bc.

SEQ ID NO:1 provides the nucleotide sequence of a cDNA encoding the murine IL-13bc. SEQ ID NO:2 provides predicted the amino acid sequence of the receptor chain, including a putative signal sequence from amino acids 1-21. The mature murine IL-13bc is believed to have the sequence of amino acids 22-383 of SEQ ID NO:2. The mature murine receptor chain has at least three distinct domains: an extracellular domain (comprising approximately amino acids 22-334 of SEQ ID NO:2), a transmembrane domain (comprising approximately amino acids 335-356 of SEQ ID NO:2) and an intracellular domain (comprising approximately amino acids 357-383 of SEQ ID NO:2).

SEQ ID NO:3 provides the nucleotide sequence of a cDNA encoding the human IL-13bc. SEQ ID NO:4 provides predicted the amino acid sequence of the receptor chain, including a putative signal sequence from amino acids 1-25. The mature human IL-13bc is believed to have the sequence of amino acids 26-380 of

SEQ ID NO:4. The mature human receptor chain has at least three distinct domains: an extracellular domain (comprising approximately amino acids 26-341 of SEQ ID NO:4), a transmembrane domain (comprising approximately amino acids 342-362 of SEQ ID NO:4) and an intracellular domain (comprising approximately amino acids 363-380 of SEQ ID NO:4).

The first 81 amino acids of the human IL-13bc sequence are identical to the translated sequence of an expressed sequence tag (EST) identified as "yg99f10.r1 Homo sapiens cDNA clone 41648 5'" and assigned database accession number R52795.gb_est2. There are no homologies or sequence motifs in this EST sequence which would lead those skilled in the art to identify the encoded protein as a cytokine receptor. A cDNA clone corresponding to this database entry is publicly-available from the I.M.A.G.E. Consortium. Subsequent to the priority date of the present application, such clone was ordered by applicants and sequenced. The sequence of such clone was determined to be the sequence previously reported by applicants as SEQ ID NO:3 herein.

Soluble forms of IL-13bc protein can also be produced. Such soluble forms include without limitation proteins comprising amino acids 1-334 or 22-334 of SEQ ID NO:2 or amino acids 1-341 or 26-341 of SEQ ID NO:4. The soluble forms of the IL-13bc are further characterized by being soluble in aqueous solution, preferably at room temperature. IL-13bc proteins comprising only the intracellular domain or a portion thereof may also be produced. Any forms of IL-13bc of less than full length are encompassed within the present invention and are referred to herein collectively with full length and mature forms as "IL-13bc" or "IL-13bc proteins." IL-13bc proteins of less than full length may be produced by expressing a corresponding fragment of the polynucleotide encoding the full-length IL-13bc protein (SEQ ID NO:1 or SEQ ID NO:3). These corresponding polynucleotide fragments are also part of the present invention. Modified polynucleotides as described above may be made by standard molecular biology techniques, including construction of appropriate desired deletion mutants, site-directed mutagenesis methods or by the polymerase chain reaction using appropriate oligonucleotide primers.

For the purposes of the present invention, a protein has "a biological activity of the IL-13 receptor binding chain" if it possess one or more of the following characteristics: (1) the ability to bind IL-13 or a fragment thereof (preferably a biologically active fragment thereof); and/or (2) the ability to interact with the second non-IL-13-binding chain of IL-13R to produce a signal characteristic of the binding of IL-13 to IL-13R. Preferably, the biological activity possessed by the protein is the ability to bind IL-13 or a fragment hereof, more preferably with a $K_D$ of about 0.1 to about 100 nM. Methods for determining whether a particular protein or peptide has such activity include without limitation the methods described in the examples provided herein.

IL-13bc or active fragments thereof (IL-13bc proteins) may be fused to carrier molecules such as immunoglobulins. For example, soluble forms of the IL-13bc may be fused through "linker" sequences to the Fc portion of an immunoglobulin. Other fusions proteins, such as those with GST, Lex-A or MBP, may also be used.

The invention also encompasses allelic variants of the nucleotide sequences as set forth in SEQ ID NO:1 or SEQ ID NO:3, that is, naturally-occurring alternative forms of the isolated polynucleotide of SEQ ID NO:1 or SEQ ID NO:3 which also encode IL-13bc proteins, preferably those proteins having a biological activity of IL-13bc. Also included in the invention are isolated polynucleotides which hybridize to the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3 under highly stringent conditions (for example, 0.1xSSC at 65°C). Isolated polynucleotides which encode IL-13bc proteins but which differ from the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3 by virtue of the degeneracy of the genetic code are also encompassed by the present invention. Variations in the nucleotide sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3 which are caused by point mutations or by induced modifications are also included in the invention.

The present invention also provides polynucleotides encoding homologues of the murine and human IL-13bc from other animal species, particularly other mammalian species. Species homologues can be identified and isolated by making

probes or primers from the murine or human sequences disclosed herein and screening a library from an appropriate species, such as for example libraries constructed from PBMCs, thymus or testis of the relevant species.

The isolated polynucleotides of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the IL-13bc protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means enzymatically or chemically ligated to form a covalent bond between the isolated polynucleotide of the invention and the expression control sequence, in such a way that the IL-13bc protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the IL-13bc protein. Any cell type capable of expressing functional IL-13bc protein may be used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D, FDCP-1, PC12, M1x or C2C12 cells.

The IL-13bc protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. Soluble forms of the IL-13bc protein may also

be produced in insect cells using appropriate isolated polynucleotides as described above.

Alternatively, the IL-13bc protein may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins.

Expression in bacteria may result in formation of inclusion bodies incorporating the recombinant protein. Thus, refolding of the recombinant protein may be required in order to produce active or more active material. Several methods for obtaining correctly folded heterologous proteins from bacterial inclusion bodies are known in the art. These methods generally involve solubilizing the protein from the inclusion bodies, then denaturing the protein completely using a chaotropic agent. When cysteine residues are present in the primary amino acid sequence of the protein, it is often necessary to accomplish the refolding in an environment which allows correct formation of disulfide bonds (a redox system). General methods of refolding are disclosed in Kohno, Meth. Enzym., 185:187-195 (1990). EP 0433225 and copending application USSN 08/163,877 describe other appropriate methods.

The IL-13bc protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a polynucleotide sequence encoding the IL-13bc protein.

The IL-13bc protein of the invention may be prepared by growing a culture transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of the IL-13bc protein of the invention can be purified from conditioned media. Membrane-bound forms of IL-13bc protein of the invention can be purified by preparing a total membrane fraction from the

expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100.

The IL-13bc protein can be purified using methods known to those skilled in the art. For example, the IL-13bc protein of the invention can be concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) or polyetheyleneimine (PEI) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred (e.g., S-Sepharose® columns). The purification of the IL-13bc protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the IL-13bc protein. Affinity columns including IL-13 or fragments thereof or including antibodies to the IL-13bc protein can also be used in purification in accordance with known methods. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein. Preferably, the isolated IL-13bc protein is purified so that it is substantially free of other mammalian proteins.

IL-13bc proteins of the invention may also be used to screen for agents which are capable of binding to IL-13bc or IL-13R or which interfere with the binding of IL-13 to the IL-13 or IL-13bc (either the extracellular or intracellular

domains) and thus may act as inhibitors of normal binding and cytokine action ("IL-13R inhibitors"). Binding assays using a desired binding protein, immobilized or not, are well known in the art and may be used for this purpose using the IL-13bc protein of the invention. Purified cell based or protein based (cell free) screening assays may be used to identify such agents. For example, IL-13bc protein may be immobilized in purified form on a carrier and binding to purified IL-13bc protein may be measured in the presence and in the absence of potential inhibiting agents. A suitable binding assay may alternatively employ a soluble form of IL-13bc of the invention. Another example of a system in which inhibitors may be screened is described in Example 2 below.

In such a screening assay, a first binding mixture is formed by combining IL-13 or a fragment thereof and IL-13bc protein, and the amount of binding in the first binding mixture ($B_o$) is measured. A second binding mixture is also formed by combining IL-13 or a fragment thereof, IL-13bc protein, and the compound or agent to be screened, and the amount of binding in the second binding mixture (B) is measured. The amounts of binding in the first and second binding mixtures are compared, for example, by performing a calculation of the ratio $B/B_o$. A compound or agent is considered to be capable of inhibiting binding if a decrease in binding in the second binding mixture as compared to the first binding mixture is observed. Optionally, the second chain of IL-13R can be added to one or both of the binding mixtures. The formulation and optimization of binding mixtures is within the level of skill in the art, such binding mixtures may also contain buffers and salts necessary to enhance or to optimize binding, and additional control assays may be included in the screening assay of the invention.

Compounds found to reduce the binding activity of IL-13bc protein to IL-13 or its fragment to any degree, preferably by at least about 10%, more preferably greater than about 50% or more, may thus be identified and then secondarily screened in other binding assays and in vivo assays. By these means compounds having inhibitory activity for IL-13bc binding which may be suitable as therapeutic agents may be identified.

IL-13bc proteins, and polynucleotides encoding them, may also be used as diagnostic agents for detecting the expression or presence of IL-13bc, IL-13R, IL-13 or cells expressing IL-13bc, IL-13R or IL-13. The proteins or polynucleotides may be employed for such purpose in standard procedures for diagnostics assays using these types of materials. Suitable methods are well known to those skilled in the art.

As used herein "IL-13R" refers to IL-13bc and/or a second IL-13 receptor chain known as "IL-13Rα1" or "NR4" (see: murine receptor chain, Hilton et al., Proc. Natl. Acad. Sci. USA 1996, 93:497-501; human receptor chain, Aman et al., J. Biol. Chem. 1996, 271:29265-70, and Gauchat et al., Eur. J. Immunol. 1997, 27:971-8).

IL-13bc acts as a mediator of the known biological activities of IL-13. As a result, IL-13bc protein (particularly, soluble IL-13bc proteins), IL-13R inhibitors (i.e., antagonists of interaction of IL-13 with IL-13R (such as, for example, antibodies to IL-13R (including particularly to IL-13bc or to IL-13Rα1) and fragments thereof, antibodies to IL-13 and fragments thereof, soluble IL-13Rα1 proteins, and small molecule and other inhibitors of the interaction of IL-13 with IL-13R (including with IL-13bc and/or with IL-13Rα1) may be useful in treatment or modulation of various medical conditions in which IL-13 is implicated or which are effected by the activity (or lack thereof) of IL-13 (collectively "IL-13-related conditions"). Mutated forms of IL-4 which bind to IL-13R can also be used as IL-13 antagonists (see, for example, those disclosed in Shanafelt et al., Proc. Natl. Acad. Sci. USA 1998, 95:9454-8; Aversa et al., J. Exp. Med. 1993, 178:2213-8; and Grunewald et al., J. Immunol. 1998, 160:4004-9).

IL-13-related conditions include without limitation Ig-mediated conditions and diseases, particularly IgE-mediated conditions (including without limitation atopy, allergic conditions, asthma, immune complex diseases (such as, for example, lupus, nephrotic syndrome, nephritis, glomerulonephritis, thyroiditis and Grave's disease)); inflammatory conditions of the lungs; immune deficiencies, specifically deficiencies in hematopoietic progenitor cells, or disorders relating thereto; cancer and other disease. Such pathological states may result from disease, exposure to

radiation or drugs, and include, for example, leukopenia, bacterial and viral infections, anemia, B cell or T cell deficiencies such as immune cell or hematopoietic cell deficiency following a bone marrow transplantation. Since IL-13 inhibits macrophage activation, IL-13bc proteins may also be useful to enhance macrophage activation (i.e., in vaccination, treatment of mycobacterial or intracellular organisms, or parasitic infections).

IL-13bc proteins may also be used to potentiate the effects of IL-13 in vitro and in vivo. For example, an IL-13bc protein can be combined with a protein having IL-13 activity (preferably IL-13) and the resulting combination can be contacted with a cell expressing at least one chain of IL-13R other than IL-13bc (preferably all chains of IL-13R other than IL-13bc, such as IL-13Rα1). Preferably, the contacting step is performed by administering a therapeutically effective amount of such combination to a mammalian subject in vivo. The pre-established association of the IL-13 protein with the IL-13bc protein will aid in formation of the complete IL-13/IL-13R complex necessary for proper signaling. See for example the methods described by Economides et al., Science 270:1351 (1995).

IL-13bc protein and IL-13R inhibitors, purified from cells or recombinantly produced, may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to IL-13bc or inhibitor and carrier, various diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration.

The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-14, IL-15, G-CSF, stem cell factor, and erythropoietin. The pharmaceutical composition may also include anti-cytokine antibodies. The pharmaceutical composition may contain thrombolytic or anti-thrombotic factors such as plasminogen activator and Factor VIII. The pharmaceutical composition may further contain other anti-

inflammatory agents. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with isolated IL-13bc protein or IL-13bc inhibitor, or to minimize side effects caused by the isolated IL-13bc or IL-13bc inhibitor. Conversely, isolated IL-13bc or IL-13bc inhibitor may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

The pharmaceutical composition of the invention may be in the form of a liposome in which isolated IL-13bc protein or IL-13bc inhibitor is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, e.g., amelioration of symptoms of, healing of, or increase in rate of healing of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of isolated IL-13bc protein or IL-13bc inhibitor is administered to a mammal. Isolated IL-13bc protein or IL-13bc inhibitor may be administered in accordance with the method of the invention either alone or in

combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, IL-13bc protein or IL-13bc inhibitor may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering IL-13bc protein or IL-13bc inhibitor in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of IL-13bc protein or IL-13bc inhibitor used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of IL-13bc protein or IL-13bc inhibitor is administered orally, IL-13bc protein or IL-13bc inhibitor will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% IL-13bc protein or IL-13bc inhibitor, and preferably from about 25 to 90% IL-13bc protein or IL-13bc inhibitor. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of IL-13bc protein or IL-13bc inhibitor, and preferably from about 1 to 50% IL-13bc protein or IL-13bc inhibitor.

When a therapeutically effective amount of IL-13bc protein or IL-13bc inhibitor is administered by intravenous, cutaneous or subcutaneous injection, IL-

13bc protein or IL-13bc inhibitor will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to IL-13bc protein or IL-13bc inhibitor an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

The amount of IL-13bc protein or IL-13bc inhibitor in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of IL-13bc protein or IL-13bc inhibitor with which to treat each individual patient. Initially, the attending physician will administer low doses of IL-13bc protein or IL-13bc inhibitor and observe the patient's response. Larger doses of IL-13bc protein or IL-13bc inhibitor may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.1 μg to about 100 mg of IL-13bc protein or IL-13bc inhibitor per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the IL-13bc protein or IL-13bc inhibitor will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

IL-13bc proteins of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the IL-13bc protein and which may inhibit binding of IL-13 or fragments thereof to the receptor. Such antibodies may be obtained using the entire IL-13bc as an immunogen, or by using fragments of IL-13bc, such as the soluble mature IL-13bc. Smaller fragments of the IL-13bc may also be used to immunize animals. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Additional peptide immunogens may be generated by replacing tyrosine residues with sulfated tyrosine residues. Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J.Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, et al., FEBS Lett. 211, 10 (1987).

Neutralizing or non-neutralizing antibodies (preferably monoclonal antibodies) binding to IL-13bc protein may also be useful therapeutics for certain tumors and also in the treatment of conditions described above. These neutralizing monoclonal antibodies may be capable of blocking IL-13 binding to the IL-13bc.

## Example 1

## Isolation of IL-13bc cDNAs

Isolation of the murine IL-13 receptor chain.

5 ug of polyA+ RNA was prepared from the thymuses of 6-8 week old C3H/HeJ mice. Double stranded, hemimethylated cDNA was prepared using Stratagene's cDNA synthesis kit according to manufacturers instructions. Briefly, the first strand was primed with an oligodT-Xho primer, and after second strand synthesis, EcoRI adapters were added, and the cDNA was digested with XhoI, and purified. The cDNA was ligated to the XhoI-EcoRI sites of the Zap Express (Stratagene) lambda vector, and packaged using Gigapak II Gold packaging extracts (Stratagene) according to the manufacturers instructions. A library of 1.5 x $10^6$ resulting recombinant phage was amplified following manufacturer's instructions. This library was screened with a degenerate 17mer oligonucleotide probe of the sequence KSRCTCCABK CRCTCCA (SEQ ID NO:5) (K = G+T; S=

C+G; R=A+G; B=C+G+T) using standard TMAC hybridization conditions as described (Current Protocols in Molecular Biology, Ausubel, et al., editors., John Wiley and Sons, 1995, section 6.4.3). Clone A25 was identified because it hybridized to the 17mer probe, but not to probes derived from known hematopoietin receptors. This clone was isolated in plasmid form from the ZapExpress vector as per manufacturers instruction, and the DNA sequence was determined. The DNA sequence encoded a novel member of the hematopoietin receptor family.

Clone A25 containing the polynucleotide having the sequence of SEQ ID NO:1 was deposited with ATCC as pA25pBKCMV at accession number 69997 on February 22, 1996.

Isolation of the human IL-13 receptor chain.

A partial fragment of the human homolog of the murine receptor was isolated by PCR using oligonucleotides derived from the murine sequence. cDNA was prepared from human testis polyA+ RNA that was obtained from Clontech. A DNA fragment of 274 base pairs was amplified from this cDNA by PCR with the following oligonucleotides: ATAGTTAAACCATTGCCACC (SEQ ID NO:6) and CTCCATTCGCTCCAAATTCC (SEQ ID NO:7) using AmpliTaq polymerase (Promega) in 1X Taq buffer containing 1.5 mM MgCl2 for 30 cycles of incubation (94°C x 1 minute, 42°C for 1 minute, and 72°C for 1 minute). The DNA sequence of this fragment was determined, and two oligonucleotides were prepared from an internal portion of this fragment with the following sequence: AGTCTATCTTACTTTTACTCG (SEQ ID NO:8) and CATCTGAGCAATAAATATTCAC (SEQ ID NO:9). These oligonucleotides were used as probes to screen a human testis cDNA library purchased from CLONTECH (cat #HL1161). Filters were hybridized at 52°C using standard 5XSSC hybridization conditions and washed in 2X SSC at 52°C. Twenty two clones were isolated that hybridized to both oligonucleotides in a screen of 400,000 clones. DNA sequence was determined from four of the cDNA clones, and all

encoded the same novel hematopoietin receptor. The predicted DNA sequence of the full length human receptor chain is shown as SEQ ID NO:3.

The human clone was deposited with ATCC as phA25#11pDR2 at accession number 69998 on February 22, 1996.

## Example 2

## Expression of Soluble IL-13bc Protein and Assay of Activity

Production and purification of soluble IL-13bc-Ig.

DNA encoding amino acids 1-331 of the extracellular domain of murine IL-13bc was fused to a spacer sequence encoding gly-ser-gly by PCR and ligated in frame with sequences encoding the hinge CH2 CH3 regions of human IgG1 of the COS-1 expression vector pED.Fc . IL-13bc-Ig was produced from DEAE-dextran transfected COS-1 cells and purified via protein A sepharose chromatography (Pharmacia).

B9 proliferation assay

Stimulation of proliferation of B9 cells (Aarden et al. Eur. J. Immunol. 1987. 17:1411-1416) in response to IL-13 or IL-4 was measured by 3H-thymidine incorporation into DNA. Cells (5 x 103/well) were seeded into 96 well plates with media containing growth factors at varying concentrations in the presence or absence of IL-13bc-Ig at 1ug/ml. After incubation for 3 days 1uCi/well of 3H-thymidine was added and the cells incubated for an additional 4 hrs. Incorporated radioactivity was determined using a LKB 1205 Plate reader.

The B9 cell line proliferated in response to IL-13, IL-4 or IL-6. Only responses to IL-13 were inhibited by the soluble IL-13bc-Ig, indicating that this receptor binds IL-13 specifically, but not IL-4 or IL-6. The tables show cpm. Two separate experiments are shown.

| cytokine dilution | IL-13 (3ng/ml) | IL-13 plus A25-Fc (1ug/ml) | IL-4 (20 ng/ml) | IL-4 plus A25-Fc (1 ug/ml) | Cos IL-6 (1/10,000) |
|---|---|---|---|---|---|
| 1 | 37734 | 1943 | 6443 | 6945 | 37887 |
| 1/3 | 30398 | 1571 | 2680 | 2442 | 36500 |
| 1/10 | 16101 | 1461 | 1767 | 1771 | 33335 |
| 1/30 | 2148 | 1567 | 1619 | 1783 | 27271 |
| 1/100 | 1574 | 1419 | 1522 | 1576 | 18831 |
| 1/300 | 1512 | 1531 | 1373 | 1577 | 7768 |
| 1/1000 | 1316 | 1392 | 1190 | 1474 | 2760 |
| 1/3000 | 1834 | 1994 | 1482 | 1819 | 1672 |

| cytokine dilution | IL-13 (3ng/ml) | IL-13 plus A25-Fc (5ug/ml) | IL-4 (20 ng/ml) | IL-4 plus A25-Fc (5ug/ml) | Cos IL-6 (1/10,000) | Cos IL-6 plus A25-Fc (5ug/ml) |
|---|---|---|---|---|---|---|
| 1 | 6413 | 295 | 1216 | 1158 | 6969 | 7703 |
| 1/3 | 5432 | 281 | 518 | 656 | 7827 | 8804 |
| 1/10 | 2051 | 281 | 489 | 520 | 8345 | 10027 |
| 1/30 | 506 | 319 | 279 | 476 | 8680 | 9114 |
| 1/100 | 430 | 372 | 288 | 423 | 7426 | 10364 |
| 1/300 | 330 | 287 | 323 | 420 | 5531 | 6254 |
| 1/1000 | 326 | 389 | 348 | nt | 2524 | nt |
| no cytokine | 339 | 279 | 404 | 394 | 326 | 279 |

Example 3

Direct Binding of Soluble IL-13bc to IL-13 Measured by Surface Plasmon Resonance (Biacore Analysis).

A Biacore biosensor was used to measure directly the specific binding of IL-13 to purified IL-13bc-Ig (Pharmacia, Johnsson et al., 1991). Approximately 10,000 to 17,000 resonance units (RU) of purified IL-13bc-Ig , human IgG1 or irrelevant receptor were each covalently immobilized to different flow cells on the sensor chip as recommended by the manufacturer. (RU's are a refelction of the mass of protein bound to the sensor chip surface.) Purified IL-13 was injected across the flow cells at 5 ul/min for 10 mins in the presence or absence of excess purified IL-13bc-Ig. Binding was quantified as the difference in RU before and after sample injection. Specific IL-13 binding of 481.9 RU was observed only for immobilized IL-13bc-Ig whereas coinjection of IL-13 plus IL-13bc-Ig resulted in no binding to the immobilized IL-13bc-Ig (4 RU). No IL-13 binding was observed for either immobilized IgG or IL-11R-Ig (5.4 and 3.7 RU respectively).

| Sample | IL-13bc-Ig (10,383 RU) | IgG control (13,399 RU) | IL-11R-Ig (17,182 RU) |
|---|---|---|---|
| 100 ng/ml human IL-13 | 481.9 RU bound | 5.4 RU bound | 3.7 RU bound |
| 100 ng/ml human IL-13 + soluble IL-13bc-Ig | 4.0 RU bound | not tested | not tested |

Example 4

Binding of IL-13 Expressed in COS Cells to

Labeled IL-13BC-Ig Fusion Protein:

COS in situ Detection of IL-13 with IL-13bc-Fc

Expression vectors for IL-13; IL-4, IL-11 or empty vector were transfected into COS-1 cells in duplicated plates via the DEAE-dextran method. Two days after transfection cells were washed twice in phosphate buffered saline (PBS) and fixed in the culture dish for 10' at 4° C with methanol. Following fixation cells were washed twice with PBS then rinsed once with binding buffer (PBS, 1% (w/v) bovine serum albumin, ).1% (w/v) sodium azide) and incubated for two hours at 4° C in binding buffer with IL-13bc-Fc at 1.0ug/ml or with relevant anti-cytokine antisera. Cells were washed twice with PBS and incubated at 4o C with shaking in alkaline phosphatase labeled Rabbit F(ab)2' anti-human IgG diluted 1:500 in binding buffer (for Fc fusion detection) or Rabbit F(ab)2' anti-rat IgG (for anti-cytokine detection). Cells were again washed twice in PBS. Alkaline phosphatase activity was visualized using nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate.

Specific binding was visualized under the microscope. Only cells transfected with IL-13 showed specific binding to IL13bc-Ig. (see photo of transfected cells, the Figure).

Example 5

Other Systems for Determination Biological Activity of IL-13bc Protein

Other systems can be used to determine whether a specific IL-13bc protein exhibits a "biological activity" of IL-13bc as defined herein. The following are examples of such systems.

Assays for IL-13 Binding

The ability of a IL-13bc protein to bind IL-13 or a fragment thereof can be determine by any suitable assays which can detect such binding. Some suitable examples follow.

Binding of IL-13 to the extracellular region of the IL-13bc protein will specifically cause a rapid induction of phosphotyrosine on the receptor protein. Assays for ligand binding activity as measured by induction of phosphorylation are described below.

Alternatively, a IL-13bc protein (such as, for example, a soluble form of the extracellular domain) is produced and used to detect IL-13 binding. For example, a DNA construct is prepared in which the extracellular domain (truncated prior, preferably immediately prior, to the predicted transmembrane domain) is ligated in frame to a cDNA encoding the hinge $C_H2$ and $C_H3$ domains of a human immunoglobulin (Ig) $\gamma 1$. This construct is generated in an appropriate expression vector for COS cells, such as pEDΔC or pMT2. The plasmid is transiently transfected into COS cells. The secreted IL-13bc-Ig fusion protein is collected in the conditioned medium and purified by protein A chromatography.

The purified IL-13bc-Ig fusion protein is used to demonstrate IL-13 binding in a number of applications. IL-13 can be coated onto the surface of an enzyme-linked immunosorbent assay (ELISA) plate, and then additional binding sites blocked with bovine serum albumin or casein using standard ELISA buffers. The IL-13bc-Ig fusion protein is then bound to the solid-phase IL-13, and binding is detected with a secondary goat anti-human Ig conjugated to horseradish peroxidase. The activity of specifically bound enzyme can be measured with a colorimetric substrate, such as tetramethyl benzidine and absorbance readings.

IL-13 may also be expressed on the surface of cells, for example by providing a transmembrane domain or glucosyl phosphatidyl inositol (GPI) linkage. Cells expressing the membrane bound IL-13 can be identified using the IL-13bc-Ig fusion protein. The soluble IL-13bc-Ig fusion is bound to the surface of these cells and detected with goat anti-human Ig conjugated to a fluorochrome, such as fluorescein isothiocyanate and flow cytometry.

Interaction Trap

A yeast genetic selection method, the "interaction trap" [Gyuris et al, Cell 75:791-803, 1993], can be used to determine whether a IL-13bc protein has a biological activity of IL-13bc as defined herein. In this system, the expression of reporter genes from both LexAop-Leu2 and LexAop-LacZ relies on the interaction between the bait protein, for example in this case a species which interacts with human IL-13bc, and the prey, for example in this case the human IL-13bc protein. Thus, one can measure the strength of the interaction by the level of Leu2 or LacZ expression. The most simple method is to measure the activity of the LacZ encoded protein, β-galactosidase. This activity can be judged by the degree of blueness on the X-Gal containing medium or filter. For the quantitative measurement of β-galactosidase activity, standard assays can be found in "Methods in Yeast Genetics" Cold Spring Harbor, New York, 1990 (by Rose, M.D., Winston, F., and Hieter, P.).

In such methods, if one wishes to determine whether the IL-13bc protein interacts with a particular species (such as, for example, a cytosolic protein which binds to the intracellular domain of the IL-13bc *in vivo*), that species can be used as the "bait" in the interaction trap with the IL-13bc protein to be tested serving as the "prey", or *vice versa*.

Example 6

Treatment of Asthma Using Soluble IL-13bc Protein

A well-characterized murine model of allergic asthma was used, in which allergen exposure leads to airway hyper responsiveness ("AHR"), pulmonary eosinophilia, elevations in antigen-specific serum IgE levels, and increases in airway epithelial mucus content (*3, 11*). Male A/J mice were immunized intraperitoneally and subsequently challenged intratracheally with soluble ovalbumin (OVA), the allergic phenotype being assessed 4 days after antigen challenge (*13*). Blockade of IL-13 was performed by the systemic administration of a soluble IL-13bc-IgGFc fusion protein (sIL-13bc-Fc), which specifically binds to and neutralizes IL-13, 24 hours before subsequent intratracheal allergen

challenge (*14*). Challenge of allergen-immunized mice resulted in significant increases in airway responsiveness to acetylcholine (*15*) (Fig. 2A). Blockade of IL-13 resulted in complete reversal of such established allergen-induced AHR; thus IL-13 is necessary for the expression of AHR in this model. The ability of IL-13 ablation to reverse AHR after full development of the phenotype of allergic asthma contrasts with the inability of IL-4 ablation to accomplish such a reversal. The mechanism underlying the effectiveness of IL-4Rα blockade in reversing allergen-induced AHR may be the inhibition of IL-13-mediated processes, consistent with the fact that Stat6 activation is downstream of IL-4Rα-mediated signaling for both cytokines. IL-13 is probably the primary CD4+ T cell-derived factor responsible for allergen-induced AHR.

To evaluate candidate mechanisms underlying IL-13-dependent expression of AHR, we characterized known allergic effector cascades. Eosinophils have been implicated as primary effector cells in asthma and asthmatic AHR (*16*), but inhibition of IL-13 prior to repeat antigen provocation did not significantly affect allergen-induced pulmonary eosinophilia *(17)* (Fig. 2B). To assess the relevance of IgE-mediated pathways, we measured OVA-specific serum IgE (*18*). OVA-specific levels of IgE were observed in OVA-sensitized and -challenged mice, whereas no antigen-specific antibody levels were detected in PBS-immunized and -challenged mice (Fig. 2C). Blockade of IL-13 did not alter OVA-specific IgE levels, a lack of suppression which is likely due to the fact that IL-13 blockade occurred after initial antigen priming and antibody formation. Nonetheless, these results show that AHR is not dependent upon IgE production in this model, consistent with reports that allergic AHR develops normally in IgE deficient and B cell deficient mice (*19*).

In congruence with the pathology of human asthma, allergic asthma in murine models is associated with a marked increase in the mucus content of the airway epithelium (*5, 11*). Mucus hypersecretion is particularly profound in autopsy specimens from patients who die of acute asthma attacks (*20*). Blockade of IL-13 reverses allergen-induced increases in mucus- containing cells in the airways (Fig. 3), demonstrating that allergen-induced increases in airway mucus content are dependent upon IL-13. IL-4 is also implicated in this process, as IL-4

transgenic mice display marked goblet cell hyperplasia in the absence of antigen sensitization (*5*). However, transfer of Th2 clones from both IL-4-deficient and control mice into murine airways induces mucus overproduction (*21*), suggesting, yet again, that the immunoregulatory role of IL-4 needs to be carefully differentiated from its role as an effector molecule.

Daily administration of recombinant IL-13 (rIL-13) to the airways of naive (unimmunized) mice induced AHR, demonstrating that increases in IL-13 activity were sufficient to induce AHR (Fig. 4A) (*22*). AHR developed by 72 hours after the start of rIL-13 administration. A significant influx of eosinophils into bronchoalveolar lavage fluid was observed early after rIL-13 administration, however pulmonary eosinophilia was not observed at the time of expression of AHR (Fig. 4B). Although the significance of the time course of eosinophil influx remains unclear, it suggests that IL-13 alone may be sufficient to initiate eosinophilic infiltration of the airways, perhaps through its ability to upregulate chemokine expression (*23*). Airway administration of rIL-13 also resulted in a time-dependent increase in total serum IgE (Fig. 4C) (*24*), in line with the previously-reported ability of IL-13 to regulate IgE synthesis (*25*). Increases in serum IgE were independent of any immunization with allergen, findings that resonate with the observation that the human asthmatic phenotype correlates better with total, rather than allergen-specific, serum IgE concentrations (*26*). As predicted from the above IL-13 inhibition studies, the administration of rIL-13 induced an increase in airway mucus production (Fig. 4D) (*27*).

**References and Notes**

1. R. M. Sly, *Ann. Allergy* 53, 20 (1984); R. Evans et al., *Chest* **91**, 65S (1987); N. Halfon and P. W. Newcheck, *Am. J. Pub. Health* **76**, 1308 (1986); R. M. Jackson, M. R. Sears, R. Beaglehole, H. H. Rea, *Chest* **94**, 914 (1988); P. J. Gergen and K. B. Weiss, *JAMA* **264**, 1688 (1990); W. M. Vollmer, A. S. Buist, M. L. Osborne, *J. Clin. Epid.* **45**, 999 (1992).

2. R. Beasley, W. R. Roche, J. A. Roberts, S. T. Holgate, *Am. Rev. Respir. Dis.* **139**, 806 (1989); R. Pauwels, *Clin. Exp. Allergy* **19**, 395 (1989); J. Bousquet et al., *N. Eng. J. Med.* **323**, 1033 (1990).

3. S. H. Gavett et al., *Am. J. Resp. Cell. Mol. Biol.* **10**, 587 (1994); A. A. Gerblich, H. Salik, M. R. Schuyler, *Amer. Rev. Resp. Dis.* **143**, 533 (1991); C. J. Corrigan, A. B. Kay, *Am. Rev. Resp. Dis.* **141**, 970 (1990); D. S. Robinson et al., *N. Engl. J. Med.* **326**, 298 (1992); C. Walker et al., *Am. Rev. Resp. Dis.* **146**, 109 (1992); S. H. Gavett et al., *J. Exp. Med.* **182**, 1527 (1995); N. W. Lukacs, R. M. Strieter, S. W. Chensue, S. L. Kunkel, *Am. J. Resp. Cell Mol. Biol.* **10**, 526 (1994).

4. F. D. Finkelman et al., *J. Immunol.* **141**, 2335 (1988); J. M. Wang et al., *Eur. J. Immunol.* **19**, 701 (1989).

5. J. A. Rankin et al., *Proc. Natl Acad. Sci. USA* **93**, 7821 (1996).

6. G. Brusselle, J. Kips, G. Joos, H. Bluethmann, R. Pauwels, *Am. J. Resp. Cell. Mol. Biol.* **12**, 254 (1995); D. B. Corry, et al., *J. Exp. Med.* **183**, 109 (1996); P. S. Foster et al., *J. Exp. Med.* **183**, 195 (1996).

7. A. J. Coyle et al., *Am. J. Resp. Cell. Mol. Biol.* **13**, 54 (1995).

8. A. K. Abbas, K. M. Murphy, A. Sher, *Nature* **383**, 787 (1996).

9. S. P. Hogan, et al., *J. Immunol.* **161**, 1501 (1998).

10. J. Punnonen et al., *Proc. Natl. Acad Sci. USA* **90**, 3730 (1993); R. de Waal Malefyt, C. G. Figdor, J. E. de Vries, *Res. Immunol.* **144**, 629 (1993); G. Zurawski, J. E. de Vries, *Immunol. Today* **15**, 19 (1994).

11. S. H. Gavett et al., *Am. J. Physiol.* **272**, L253 (1977); D. Kuperman, B. Schofield, M. Wills-Karp, M. J. Grusby, *J. Exp. Med.* **187**, 939 (1998).

12. S. M. Zurawski, G. Zurawski, *EMBO J.* **11**, 3905 (1993); S. M. Zurawski et al., *J. Biol. Chem.* **270**, (1995). J.-X. Lin et al., *Immunity* **2**, 331 (1995).

13. Six-week-old male A/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and were housed under laminar flow hoods in an environmentally-controlled specific pathogen-free animal facility for the duration of experiments (N = 4-10 mice/experimental group). The studies reported here conformed to the principles for laboratory animal research outlined by the Animal Welfare Act and the Department of Health, Education and Welfare (N.I.H.) guidelines for the experimental use of animals. Mice were immunized by an intraperitoneal injection of 10 ug ovalbumin (OVA; Crude grade IV, Sigma; St. Louis, MO) in 0.2 ml PBS or PBS alone. 14 days after immunization, mice were anesthetized with a mixture of ketamine and xylazine (45 and 8 mg/kg,

respectively) and challenged intratracheally with 50 ul of a 1.5% solution of OVA or an equivalent volume of PBS as a control. 10 days after this first antigen challenge, mice were challenged again intratracheally with either OVA or PBS. Characterization of the allergic phenotype was performed 96 hours after the second antigen challenge.

14. Human IL-13bc was cloned as described above. For soluble expression of the murine homolog, a pED expression vector containing DNA encoding the murine sIL-13bc extracellular domain, fused in frame with the hinge CH2/CH3 regions of human IgG1 (as described in previous examples), was transfected into CHO cells [D. D. Donaldson et al., *J. Immunol.* **161**, 2317 (1998)]. The sIL-13bc-Fc was purified with rProtein A-Sepharose [J. F. Urban et al., *Immunity* **8**, 255 (1998)]. The in vitro $ID_{50}$, as determined by the ability to neutralize 3 ng/ml of murine IL-13 in the B9 proliferation assay was approximately 10 ng/ml. Human IgG, used as a control for sIL-13bc-Fc, was similarly purified by rProtein A-Sepharose chromatography from a 10% solution of human immune globulin that is commercially available for intravenous administration (Miles) [*ibid*]. Mice were given sIL-13bc-Fc (400ug), or an equivalent amount of the control hu-IgG, by intraperitoneal injection on Day -1, O, +1, and +3 of secondary antigen challenge.

15. Airway reactivity to intravenous administration of acetylcholine was measured (*11*), 3 days after final intratracheal challenge. Mice were anesthetized with sodium pentobarbital (90 mg/kg), intubated, ventilated at a rate of 120 breaths/minute with a constant tidal volume of air (0.2 ml), and paralyzed with decamethonium bromide (25 mg/kg). After establishment of a stable airway pressure, acetylcholine was injected intravenously (50 ug/kg) and dynamic airway pressure was followed for 5 minutes.

16. G. J. Gleich, *J. All. Clin. Immunol.* **8**,422 (1990).

17. Bronchoalveolar lavage was conducted as described (*11*).

18. A kidney was excised, and pooled blood was collected for antibody analysis as described (*11*). Serum was separated by centrifugation and stored at -80°C until analysis. Serum OVA-specific IgE levels were determined by sandwich ELISA . Sample wells were coated with a 0.01% OVA solution in PBS, blocked with 10% FBS in PBS, and washed with 0.05% Tween-20 in PBS. Serum samples were

diluted 1:10 and 1:100 with 10% FBS in PBS. After an overnight incubation, plates were washed with 0.05% Tween-20 in PBS and biotin-conjugated anti-mouse IgE (PharMingen, San Diego, CA) was added. After a wash, 0.0025 mg/ml avidin peroxidase (Sigma) in 10% FBS/PBS was added, and plates were developed with ABTS (2.2'-azino-did[3-ethyl-benzthiazone sulfonate]) (Kirkegaard and Perry). Plates were read at 405 nm within 30 minutes. Reported 0.D. values are of serum samples diluted 1:10 since these values were proven to be below the saturation point of the assay by comparison of O.D. values of serum samples diluted 1:100 with 10% FBS/PBS.

19. P. D. Mehlhop et al., *Proc. Natl. Acad. Sci. USA* **94**, 1344 (1997); **M. Korsgren et al., *J. Exp. Med.* 185, 885 (1997).**

20. T. Aikawa et al., *Chest* **101**, 916 (1992).

21. L. Cohn, R. J. Homer, A. Marinov, J. Rankin, K. Bottomly. *J. Exp. Med.* **186**, 1737 (1997).

22. DNA encoding a honeybee melittin leader [D. C. Tessier, D. Y. Thomas, H. E. Khouri, F. Laliberte, T. Vernet, *Gene* **2**, 177 (1991)] followed by a six-histidine tag was fused by an enterokinase cleavage site to the mature region of murine IL-13 at Gly21 and constructed in the mammalian expression vector pHTop. H6-EK murine IL-13 protein was produced from stably- transfected CHO cells and purified via Ni-NTA chromatography to greater than 97% purity as determined by SDS-PAGE. Protein concentration was determined by absorption at 280 nm and endotoxin contamination was less than 30 EU/mg as measured by Cape Cod Associates LAL assay. The $ED_{50}$ of H6-EK murine IL-13 as determined by the Ba/F3.IL-13R 1 proliferation assay was 1ng/ml. Murine rIL-13 (5ug in a total volume of 50ul) was administered daily by intratracheal instillation to naive mice anesthesized with a mixture of ketamine and xylazine (45 and 8 mg/kg, respectively).

23. M. Goebeler et al., *Immunol.* **91**, 450 (1997).

24. A murine IgE-specific ELISA was used to quantitate total IgE immunoglobulin levels in serum using complementary antibody pairs for mouse IgE (R35-72 and R35-92) obtained from PharMingen according to the manufacturer's instructions. Duplicate samples (of a 1/10 dilution in 10% FBS in

PBS) were examined from each animal. O.D. readings of samples were converted to pg/ml using values obtained from standard curves generated with known concentrations of recombinant mouse IgE (5-2000 pg/ml), and the final concentration was obtained by multiplying by the dilution factor.

25. C. L. Emson, S. E. Bell, A. Jones, W. Wisden, A. N. J. McKenzie, *J. Exp. Med.* **188**, 399 (1998).

26. L. R. Friedhoff, D. G. Marsh, *Int. Arch. All. Immunol.* **100**, 355 (1993).

27. To examine the effects of rIL-13 on mucus cell content of the airway epithelium, lungs were excised and fixed in 10% formalin. They were then washed in 70% ethanol, dehydrated, embedded in glycol methacrylate, cut into 10 uM sections, mounted on slides, and stained with hematoxylin and eosin and periodic acid Schiff. Four sections were examined per animal; 4 fields were scored per lung section. Sections were scored on a scale from 1-4 with 1 representing no mucus cell content.

28. J. Luyimbazi, X. Xu, M. Wills-Karp, unpublished results.

29. C. Walker et al., *Am. Rev. Respir. Dis.* **146**, 109 (1992); M. Humbert et al., *J. All. Clin. Immunol.* **99**, 657 (1997); S. K. Huang, *J. Immunol.* **155**, 2688 (1995).

30. D. G. Marsh, et al., *Science* **264**, 1152 (1996).

31. L. J. Rosenwasser. *N. Engl. J. Med.* **337**, 1766 (1977).

32. G. K. Hershey et al., *N Engl. J. Med.* 337, 1720 (1997).

All patent and literature references cited herein are incorporated by reference as if fully set forth.

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:

(a) the nucleotide sequence of SEQ ID NO:1 from nucleotide 256 to nucleotide 1404;

(b) the nucleotide sequence of SEQ ID NO:3 from nucleotide 103 to nucleotide 1242;

(c) a nucleotide sequence varying from the sequence of the nucleotide sequence specified in (a) or (b) as a result of degeneracy of the genetic code;

(d) a nucleotide sequence capable of hybridizing under stringent conditions to the nucleotide specified in (a) or (b);

(e) a nucleotide sequence encoding a species homologue of the sequence specified in (a) or (b); and

(f) an allelic variant of the nucleotide sequence specified in (a) or (b).

2. The polynucleotide of claim 1 wherein said nucleotide sequence encodes for a protein having a biological activity of the IL-13R binding chain.

3. The polynucleotide of claim 1 wherein said nucleotide sequence is operably linked to an expression control sequence.

4. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 319 to nucleotide 1257.

5. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 1324 to nucleotide 1404.

6. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 178 to nucleotide 1125.

7. The polynucleotide of claim 1 comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 1189 to nucleotide 1242.

8. A host cell transformed with the polynucleotide of claim 3.

9. The host cell of claim 8, wherein said cell is a mammalian cell.

10. A process for producing a IL-13bc protein, said process comprising:

(a) growing a culture of the host cell of claim 8 in a suitable culture medium; and

(b) purifying the IL-13bc protein from the culture.

11. An isolated IL-13bc protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 334;

(c) the amino acid sequence of SEQ ID NO:2 from amino acids 357 to 383;

(d) the amino acid sequence of SEQ ID NO:4;

(e) the amino acid sequence of SEQ ID NO:4 from amino acids 26 to 341;

(f) the amino acid sequence of SEQ ID NO:4 from amino acids 363 to 380; and

(g) fragments of (a)-(f) having a biological activity of the IL-13 receptor binding chain.

12. The protein of claim 11 comprising the amino acid sequence of SEQ ID NO:2.

13. The protein of claim 11 comprising the sequence from amino acid 22 to 334 of SEQ ID NO:2.

14. The protein of claim 11 comprising the amino acid sequence of SEQ ID NO:4.

15. The protein of claim 11 comprising the sequence from amino acid 26 to 341 of SEQ ID NO:4.

16. A pharmaceutical composition comprising a protein of claim 11 and a pharmaceutically acceptable carrier.

17. A protein produced according to the process of claim 10.

18. A composition comprising an antibody which specifically reacts with a protein of claim 11.

19. A method of identifying an inhibitor of IL-13 binding to the IL-13 receptor which comprises:

(a) combining a protein of claim 11 with IL-13 or a fragment thereof, said combination forming a first binding mixture;

(b) measuring the amount of binding between the protein and the IL-13 or fragment in the first binding mixture;

(c) combining a compound with the protein and the IL-13 or fragment to form a second binding mixture;

(d) measuring the amount of binding in the second binding mixture; and

(e) comparing the amount of binding in the first binding mixture with the amount of binding in the second binding mixture;

wherein the compound is capable of inhibiting IL-13 binding to the IL-13 receptor when a decrease in the amount of binding of the second binding mixture occurs.

20. An inhibitor identified by the method of claim 19.

21. A pharmaceutical composition comprising the inhibitor of claim 20 and a pharmaceutically acceptable carrier.

22. A method of inhibiting binding of IL-13 to the IL-13 receptor in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition of claim 21.

23. A method of inhibiting binding of IL-13 to the IL-13 receptor in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition of claim 16.

24. A method of inhibiting binding of IL-13 to the IL-13 receptor in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition of claim 18.

25. An isolated polynucleotide comprising a nucleotide sequence encoding a peptide or protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:2 from amino acids 22 to 334;

(c) the amino acid sequence of SEQ ID NO:2 from amino acids 357 to 383;

(d) the amino acid sequence of SEQ ID NO:4;

(e) the amino acid sequence of SEQ ID NO:4 from amino acids 26 to 341;

(f) the amino acid sequence of SEQ ID NO:4 from amino acids 363 to 380; and

(g) fragments of (a)-(f) having a biological activity of the IL-13 receptor binding chain.

26. The protein of claim 11 wherein said amino acid sequence is part of a fusion protein.

27. The protein of claim 26 comprising an Fc fragment.

28. A method of treating an IL-13-related condition in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition of claim 16.

29. The method of claim 28 wherein said condition is an IgE-mediated condition.

30. The method of claim 29 wherein said condition is selected from the group consisting of atopy, an allergic condition, asthma and an immune complex disease.

31. The method of claim 30 wherein said condition is selected from the group consisting of lupus, nephritis, thyroiditis and Grave's disease.

32. A method for potentiating IL-13 activity, said method comprising combining a protein having IL-13 activity with a protein of claim 11 and contacting such combination with a cell expressing at least one chain of IL-13R other than IL-13bc.

33. The method of claim 32 wherein the contacting step is performed by administering a therapeutically effective amount of such combination to a mammalian subject.

34. The protein of claim 11 comprising the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 331.

35. The protein of claim 11 comprising the amino acid sequence of SEQ ID NO:2 from amino acids 26 to 331.

36. The polynucleotide of claim 25 encoding a peptide or protein comprising the amino acid sequence of SEQ ID NO:2 from amino acids 1 to 331

37. The polynucleotide of claim 25 encoding a peptide or protein comprising the amino acid sequence of SEQ ID NO:2 from amino acids 26 to 331.

38. The method of claim 28 wherein said condition is an inflammatory condition of the lung.

39. A method of treating an IL-13-related condition in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition comprising an IL-13 antagonist and a pharmaceutically acceptable carrier.

40. The method of claim 39 wherein said condition is an IgE-mediated condition.

41. The method of claim 40 wherein said condition is selected from the group consisting of atopy, an allergic condition, asthma and an immune complex disease.

42. The method of claim 41 wherein said condition is selected from the group consisting of lupus, nephritis, thyroiditis and Grave's disease.

43. The method of claim 39 wherein said antagonist is selected from the group consisting of an IL-13bc protein, a soluble form of IL-13Rα1, an antibody to IL-13 or an IL-13-binding fragment thereof, an antibody to IL-13bc or an IL-13bc-binding fragment thereof, an antibody to IL-13Rα1 or an IL-13Rα1-binding fragment thereof, IL-13R-binding mutants of IL-4, a small molecule capable of inhibiting the interaction of IL-13 with IL-13bc and a small molecule capable of inhibiting the interaction of IL-13 with IL-13Rα1.

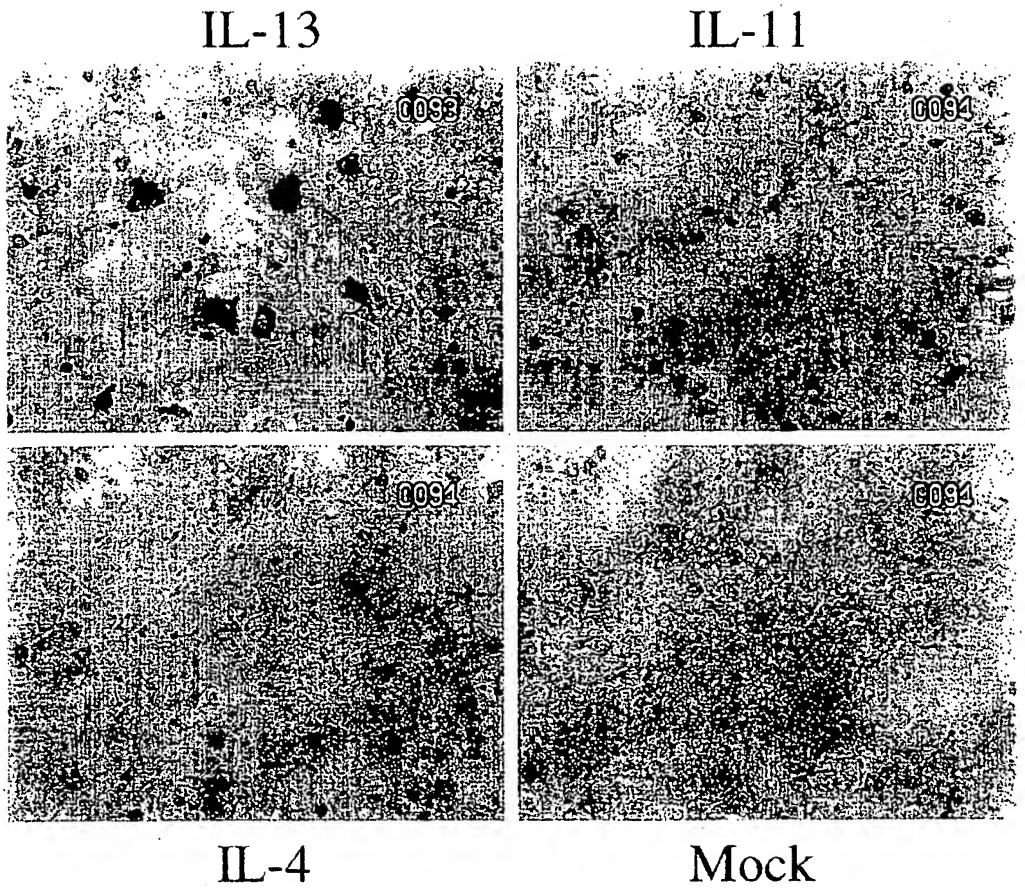
44. The method of claim 43 wherein said IL-13bc protein is a protein of claim 11.

45. A method of inhibiting the interaction of IL-13 with an IL-13bc protein in a mammalian subject, said method comprising administering a therapeutically effective amount of a composition comprising an IL-13 antagonist and a pharmaceutically acceptable carrier.

46. The method of claim 45 wherein said antagonist is selected from the group consisting of an IL-13bc protein, a soluble form of IL-13Rα1, an antibody to IL-13 or an IL-13-binding fragment thereof, an antibody to IL-13bc or an IL-13bc-binding fragment thereof, an antibody to IL-13Rα1 or an IL-13Rα1-binding fragment thereof, IL-13R-binding mutants of IL-4, a small molecule capable of inhibiting the interaction of IL-13 with IL-13bc and a small molecule capable of inhibiting the interaction of IL-13 with IL-13Rα1.

47. The method of claim 46 wherein said IL-13bc protein is a protein of claim 11.

Figure 1



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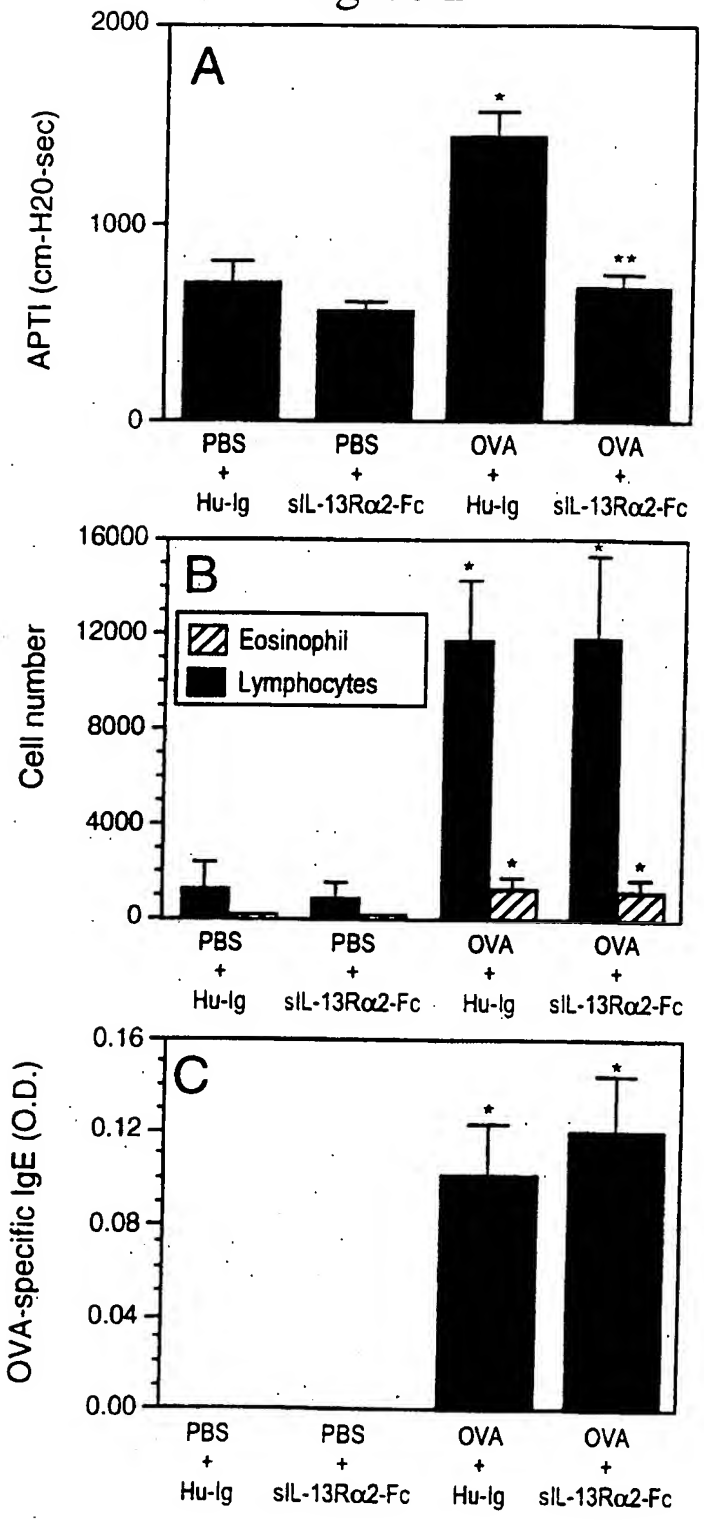
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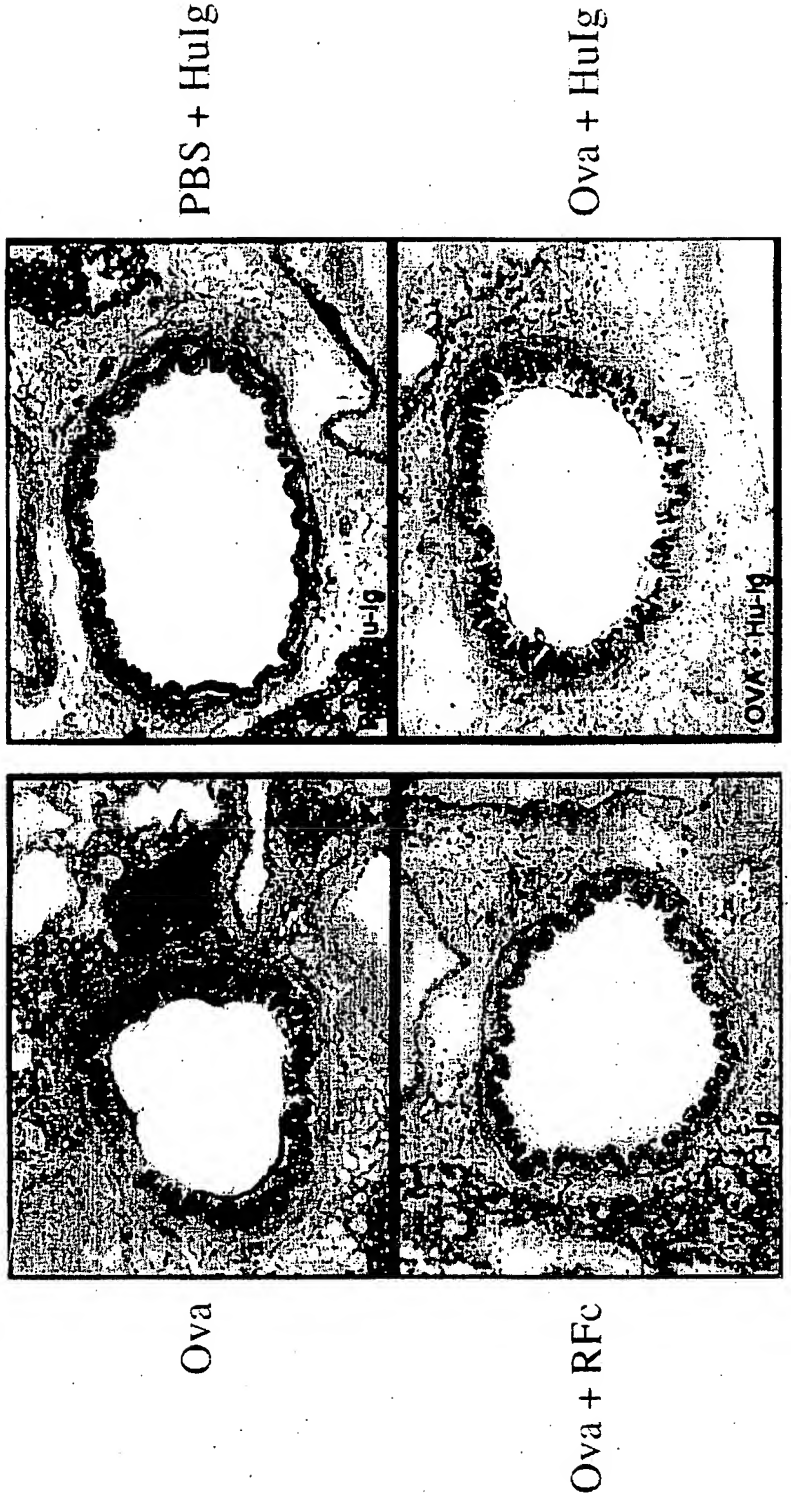

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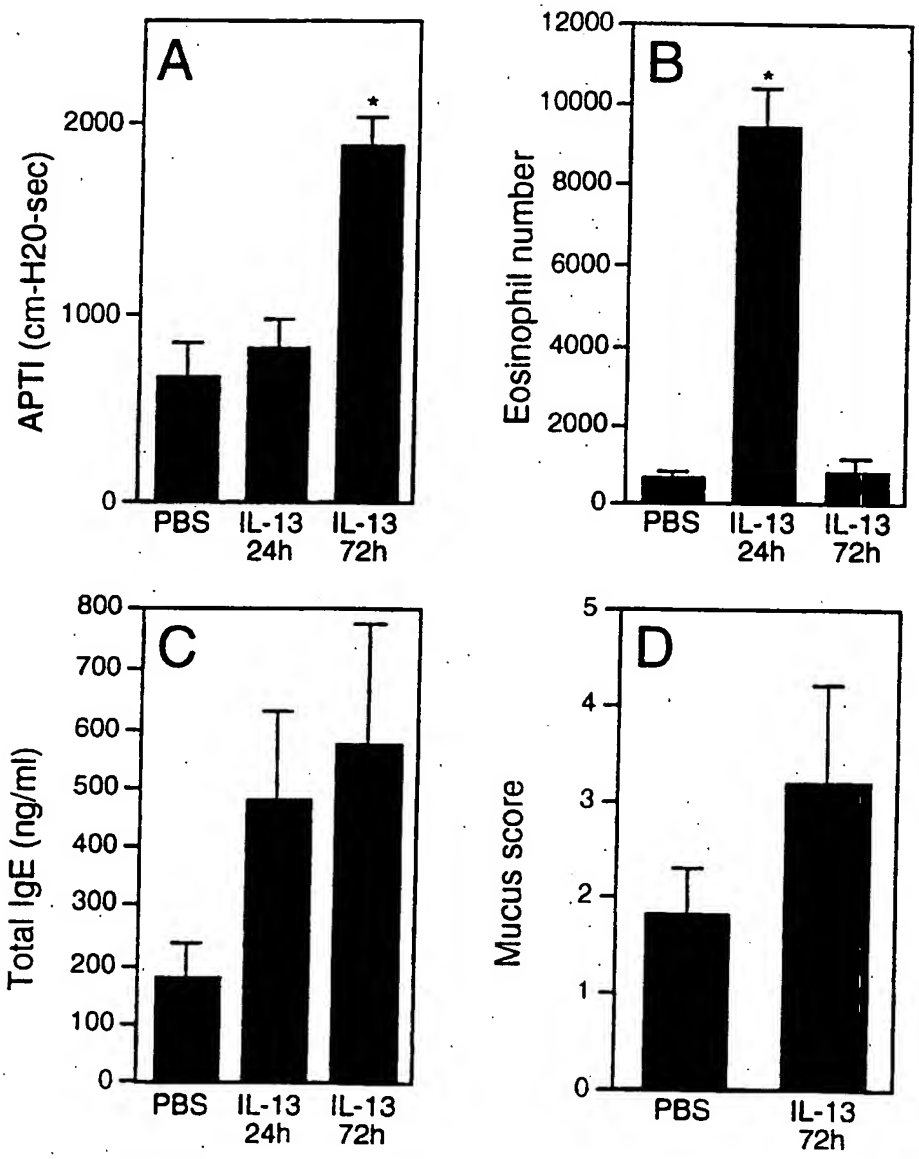
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Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr Trp Ile Ser Pro
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caa gga att cca gaa act aaa gtt cag gat atg gat tgc gta tat tac   546
Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp Cys Val Tyr Tyr
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Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly Ile Gly Val Leu
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ctt gat acc aat tac aac ttg ttt tac tgg tat gag ggc ttg gat cat   642
Leu Asp Thr Asn Tyr Asn Leu Phe Tyr Trp Tyr Glu Gly Leu Asp His
165                 170                 175                 180

gca tta cag tgt gtt gat tac atc aag gct gat gga caa aat ata gga   690
Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly Gln Asn Ile Gly
                185                 190                 195

tgc aga ttt ccc tat ttg gag gca tca gac tat aaa gat ttc tat att   738
Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys Asp Phe Tyr Ile
            200                 205                 210

tgt gtt aat gga tca tca gag aac aag cct atc aga tcc agt tat ttc   786
Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg Ser Ser Tyr Phe
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act ttt cag ctt caa aat ata gtt aaa cct ttg ccg cca gtc tat ctt   834
Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro Pro Val Tyr Leu
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act ttt act cgg gag agt tca tgt gaa att aag ctg aaa tgg agc ata   882
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Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr Glu Ile Glu Ile
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aga gaa gat gat act acc ttg gtg act gct aca gtt gaa aat gaa aca   978
Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val Glu Asn Glu Thr
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tac acc ttg aaa aca aca aat gaa acc cga caa tta tgc ttt gta gta   1026
Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu Cys Phe Val Val
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aga agc aaa gtg aat att tat tgc tca gat gac gga att tgg agt gag   1074
Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly Ile Trp Ser Glu
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tgg agt gat aaa caa tgc tgg gaa ggt gaa gac cta tcg aag aaa act   1122
Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu Ser Lys Lys Thr
325                 330                 335                 340

ttg cta cgt ttc tgg cta cca ttt ggt ttc atc tta ata tta gtt ata   1170
Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu Ile Leu Val Ile
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ttt gta acc ggt ctg ctt ttg cgt aag cca aac acc tac cca aaa atg   1218
Phe Val Thr Gly Leu Leu Leu Arg Lys Pro Asn Thr Tyr Pro Lys Met
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att cca gaa ttt ttc tgt gat aca tga agactttcca tatcaagaga         1265
Ile Pro Glu Phe Phe Cys Asp Thr
        375                 380

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Leu Tyr Leu Gln Trp Gln Pro Pro Leu Ser Leu Asp His Phe Lys Glu
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Cys Thr Asn Gly Ser Glu Val Gln Ser Ser Trp Ala Glu Thr Thr Tyr
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Trp Ile Ser Pro Gln Gly Ile Pro Glu Thr Lys Val Gln Asp Met Asp
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Cys Val Tyr Tyr Asn Trp Gln Tyr Leu Leu Cys Ser Trp Lys Pro Gly
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Gly Leu Asp His Ala Leu Gln Cys Val Asp Tyr Ile Lys Ala Asp Gly
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Gln Asn Ile Gly Cys Arg Phe Pro Tyr Leu Glu Ala Ser Asp Tyr Lys
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Asp Phe Tyr Ile Cys Val Asn Gly Ser Ser Glu Asn Lys Pro Ile Arg
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Ser Ser Tyr Phe Thr Phe Gln Leu Gln Asn Ile Val Lys Pro Leu Pro
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Pro Val Tyr Leu Thr Phe Thr Arg Glu Ser Ser Cys Glu Ile Lys Leu
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Lys Trp Ser Ile Pro Leu Gly Pro Ile Pro Ala Arg Cys Phe Asp Tyr
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Glu Ile Glu Ile Arg Glu Asp Asp Thr Thr Leu Val Thr Ala Thr Val
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Glu Asn Glu Thr Tyr Thr Leu Lys Thr Thr Asn Glu Thr Arg Gln Leu
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Cys Phe Val Val Arg Ser Lys Val Asn Ile Tyr Cys Ser Asp Asp Gly
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Ile Trp Ser Glu Trp Ser Asp Lys Gln Cys Trp Glu Gly Glu Asp Leu
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Ser Lys Lys Thr Leu Leu Arg Phe Trp Leu Pro Phe Gly Phe Ile Leu
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# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29493

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) :Please See Extra Sheet.
US CL :435/69.51, 471, 252.3, 325, 7.1; 530/351, 389.1; 424/85.1
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.51, 471, 252.3, 325, 7.1; 530/351, 389.1; 424/85.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

West, US Patent full, STN via medline, caplus, embase and biosis.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>------<br>Y | WO 97/15663 A1 (AMRAD OPERATIONS PTY LTD.) 01 May 1997 (01.05.97), see especially page 3, lines 19-25; page 17, lines 23-27; page 28, lines 6-11; page 31, lines 1-2; page 22, lines 5-11; page 38, lines 1-15; page 18, lines 1-14 and claims. | 1-30, 33-37, 39-41,45<br>----------<br>31, 32, 38, 42-44, 46-47 |
| A | US 5,696,234 A (ZURAWSKI et al) 09 December 1997 (09.12.97), see entire document. | 1-47 |
| A | US 5,599,905 A (MOSLEY et al) 04 February 1997 (04.02.97), see entire document. | 1-47 |
| A | OBIRI et al. Receptor for Interleukin 13: Interaction with interleukin 4 by a mechanism that does not involve the common γ chain shared by receptors for interleukins 2, 4, 7, 9 and 15. The Journal of Biological Chemistry. 14 April 1995, Vol. 270, No. 15, pages 8797-8804, see entire document. | 1-47 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 07 APRIL 2000 | 25 APR 2000 |

| Name and mailing address of the ISA/US | Authorized officer |
|---|---|
| Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231 | FOZIA HAMUD<br>JOYCE BRIDGERS<br>PARALEGAL SPECIALIST<br>CHEMICAL MATRIX |
| Facsimile No. (703) 305-3230 | Telephone No. (703) 308-0196 |

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29493

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | ZURAWSKI et al. The Primary Binding Subunit of the Human Interleukin-4 Receptor Is Also a Component of the Interleukin-13 Receptor. The Journal of Biological Chemistry. 09 June 1995, Vol. 270, No. 23, pages 13869-13878, see entire document. | 1-47 |
| A | VITA et al. Characterization and Comparison of the Interleukin 13 Receptor with the Interleukin 4 Receptor on Several Cell Types.The Journal of Biological Chemistry. 24 February 1995, Vol. 270, No. 8, pages 3512-3517, see entire document. | 1-47 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/29493

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C12N 15/12, 15/19, 5/00, 5/10; C07K 14/47, 14/52; A16K 38/17, 38/20, 48/00